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E-mail:xbywb@ouc.edu.cn

Effects of Temperature, Salinity and Stocking Density on Larval Survival and Growth of Reciprocal Crosses Between Two Strains of Pacific Oysters, *Crassostrea gigas*

MENG Lingxin[1)], XU Chengxun[1)], and LI Qi[1), 2), *]

1) *Key Laboratory of Mariculture*, *Ministry of Education*, *Ocean University of China*, *Qingdao* 266003, *China*
2) *Laboratory for Marine Fisheries Science and Food Production Processes*, *Pilot National Laboratory for Marine Science and Technology (Qingdao)*, *Qingdao* 266237, *China*

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Abstract The Pacific oyster *Crassostrea gigas* is one of the most widely cultivated aquaculture species and contributes significantly to total seafood production for human beings. However, mass mortality occurred frequently, and in some regions almost all oysters died during seed production and grow-out stage. In order to explore whether hybridization breeding can improve its growth and survival, a complete diallel cross between a selected strain 'Haida No. 1' (S) and an orange shell variant (O) of *C. gigas* was carried out. The larval growth and survival were compared among hybrids and purebred strains at temperatures of 16, 20, 24, 28 and 32℃; salinities of 15, 20, 25, 30 and 35; and stocking densities of 0.5, 1, 2, 4 and 8 larvae mL^{-1}. Under different environments, the hybridization between two strains of *C. gigas* showed the heterosis of growth and survival. The mean shell height and survival rate of the two reciprocal crosses (OS, SO) were significantly higher than those of the two purebred strains (SS, OO) under all environmental conditions. In particular, OS showed greater heterosis than the purebred strains and SO progeny. The results showed that the productive traits of the 'Haida No. 1' could be improved by crossing with the orange shell line. Meanwhile, the results from this study also indicated that hybridization between the two strains of *C. gigas* may be a promising way for breeding new variety with high survival rate.

Key words *Crassostrea gigas*; heterosis; larvae; temperature; salinity; stocking density; survival; growth

1 Introduction

Oysters are important species in shellfish industry in China, accounting for >30% of the total marine mollusc yield with a total production of 5.2 million tons in 2019 (DOF, 2020). The Pacific oyster (*Crassostrea gigas*) is one of the most widely cultivated oyster species in northern China. Due to its high commercial value, a number of selective breeding programs of *C. gigas* based on family or mass selection have been launched, leading to significant improvements of commercially important traits, such as growth rate (Li *et al*., 2011; Xu *et al*., 2019) and shell coloration (Han *et al*., 2019). Despite these efforts, mass mortality has occurred frequently. In some regions, almost all stocks died during seed production and grow-out stage. A large-scale summer mortality of Pacific oysters has become a major problem affecting oyster aquaculture. Hybridization breeding is regarded as an effective method to transfer desirable traits between species, breed new varieties, increase environmental tolerance, and produce genetic improvement of marine shellfish (Hedgecock *et al*., 1995; Hedgecock and Davis, 2007). A number of hybridization experiments have been conducted in marine bivalves, such as scallops (Zheng *et al.*, 2011), mussels (Matson *et al.*, 2003) and oysters (Tan *et al*., 2020).

In *C. gigas*, efforts have been made to produce reciprocal crosses through interspecific and intraspecific hybridization. Because of the genetic incompatibility between different species, only a few studies demonstrated that the beneficial development traits of *C. gigas* can be achieved by interspecific hybridization (Xu *et al*., 2019; Tan *et al*., 2020). On the other hand, more attentions have been focused on the intraspecific hybridization, which generally has higher hatching rate and growth performance compared with interspecific hybridization (Hulata, 2001). Our previous studies proved that the high heterosis for growth and survival of *C. gigas* can be obtained by crossing among different breeding lines (Kong *et al*., 2017; Han *et al*., 2020). However, most of these investigations put particular emphasis on comparing the differences among hybrids under the identical condition, did not examine the performance in the growth and survival of hybrid progeny under different environments. In order to obtain an accurate evaluation of the performance for hybrids, the complex environmental conditions need to be considered.

* Corresponding author. E-mail: qili66@ouc.edu.cn

The pelagic larval phase is the most vulnerable stage. The growth and survival of the larvae at this stage are possibly most susceptible to the external environmental conditions, such as temperature, salinity and stocking density (Claudi and Evans, 1993). Water temperature and salinity are selected by many researchers to study their effects on bivalve ontogeny (Li and Li, 2010; Xu *et al*., 2019). The fluctuation of temperature and salinity has become major stress factors for many marine organisms in various ways, such as growth performance (Mak and Chan, 2018), disease resistance (Fuhrmann *et al*., 2016), physiological and biochemical responses (Pourmozaffar *et al*., 2019), geographic distribution (Matsuda *et al*., 2008), and food consumption (Wasielesky *et al*., 2003). In addition to the temperature and salinity, one of the principle variables in hatchery conditions is stocking density, which is also an important factor for cultivation-related economic variability (Ren *et al*., 2017). Inappropriate larval stocking density can lead to suboptimal growth and survival performance. Thus, favorable stocking density for *C. gigas* cultivation is necessary in terms of maintaining a positive correlation between density and growth rate.

In our previous study, we have bred a selected strain 'Haida No. 1' with rapid growth characteristics and an inbreeding line with orange shell color. In the present study, two strains of *C. gigas* and their reciprocal crosses were evaluated to determine if heterosis exists in the productive characteristics such as growth and survival during larval stage, and compare the heterosis under different temperatures, salinities and stocking densities.

2 Materials and Methods

2.1 Broodstock Collection and Larval Rearing

One-year-old *C. gigas* were collected from a selected strain 'Haida No. 1' (S: 12th generation) and an orange shell variant (O: 9th generation). The broodstock of both strains were grown in the same area located in Rushan, Shandong Province, China (36.4°N, 121.3°E). Both strains were transferred to Laizhou Haiyi hatchery, Shandong Province, and a pool with 24 m^3 of seawater (temperature: 24℃; salinity: 30) was used for 1 month of acclimation prior to experiment. Fifty sexually matured *C. gigas* from each strain were induced to spawn. A complete diallel cross produced four experimental groups, including two purebred strains of SS (S♀×S♂) and OO (O♀×O♂), and two reciprocal hybrids of OS (O♀×S♂) and SO (S♀×O♂).

After 24-h incubation, D-larvae were collected and transferred into 15-L rearing tanks. A total of 180 tanks were used in the experiment (15 treatments × 4 strains × 3 replicates). Larvae were fed excessively with *Isochrysis galbana* three times a day. For the temperature and salinity experiment, the concentration of 15×10^3 cells mL^{-1} was maintained until the end of the experiment. And for the stocking density experiment, the concentration of *I. galbana* proportionately increased with stocking density to supply larvae in each treatment group with the same amount of algae (Table 1). Before the algae were added to each container, same volume of water was removed to maintain the constant water volume.

Table 1 Daily algal ratio ($\times10^3$ cells mL^{-1}) for *C. gigas* larvae reared under different stocking densities during the experimental period

Experimental period	Stocking density (larval mL^{-1})				
	0.5	1	2	4	8
Days 1–4	1.5	3.0	6.0	12.0	24.0
Days 5–8	2.5	5.0	10.0	20.0	40.0
Days 9–12	4.0	8.0	16.0	32.0	64.0
Days 13–16	6.0	12.0	24.0	48.0	96.0

During the rearing period, larvae containers were slightly aerated to increase the oxygen concentration and reduce the accumulation of organic matter. Every second day morning, 100% of the water was renewed. The fresh seawater was filtered through sand and nonwoven polypropylene fabric and adjusted to the test condition before changing. During water changes, the larvae were transferred onto a 55-μm mesh and gently washed back into containers after the containers had been lightly scrubbed and refilled with fresh seawater.

2.2 Experimental Design

Five experimental temperatures of 16, 20, 24, 28 and 32℃ were selected to evaluate the effect of temperature change on growth and survival of *C. gigas* larvae. The salinity was kept stable at 30 and the initial larval density was kept at 2 larvae mL^{-1}. Water temperature was maintained by water bath with immersed heaters or water chiller (HC-150A, 33ILEA, China) and a temperature regulator. Water temperature was gradually adjusted to the experimental requirements at a rate of 0.5–1℃ per hour. Five experimental salinities of 15, 20, 25, 30 and 35 were established to examine the effects of salinity change on growth and survival of larvae. The temperature was kept stable at 24℃ and the initial larval density was kept at 2 larvae mL^{-1}. Water salinity was adjusted by diluting natural seawater with filtered fresh water (filtered through a 50-μm mesh sieve) or adding sea salt and measured by an optical salinometer. The initial salinity was raised or lowered at a rate of 2 until desired salinity was obtained. The effects of stocking densities were examined at five different densities (0.5, 1, 2, 4, and 8 larvae mL^{-1}). The rearing temperature and salinity were maintained at 24℃ and 30, respectively.

2.3 Measurement and Analysis

In the temperature and salinity experiment, a 50-mL sample was collected randomly. In the stocking density experiment, 200-, 100-, 50-, 25- and 10-mL samples were randomly taken from containers with stocking densities of 0.5, 1, 2, 4 and 8 larvae mL^{-1}. Subsequently, the samples were fixed by the addition of 1% Lugol's solution to determine the mean shell height and survival. The shell height of 30 larvae randomly selected in each replicate sample were quantified using a light microscope (160×) equipped with an ocular micrometer. Larval survival was based on an ini-

tial density. Larvae were sampled three times to calculate survival rate for each replicate, and the water volume was adjusted according to the survival rate to ensure that each experimental tank always meets the required cultivation density.

The data were analyzed using SPSS 20.0 (IBM SPSS Inc., Chicago, IL, USA). The differences of shell height and survival rate among different strains were analyzed using one-way analysis of variance (ANOVA), followed by the least significant difference (LSD) test and Duncan's multiple range tests for *post hoc* mean comparisons. Differences were considered statistically significant if $P<0.05$.

Heterosis was calculated to evaluate the aquaculture traits, the equation to determine mid-parent heterosis (H) was taken from Falconer and Mackay (1996):

$$H(\%)=\left[(F_1-P)\times 100\right]/P,$$

where P is the average phenotypic value of the two parental populations; F_1 is the mean value of one hybrid cross.

3 Results

3.1 Effects of Temperature on Larval Growth and Survival

A significant difference in the shell height appeared starting from day 8 ($P<0.05$), and the shell heights of four strains were the largest at 28℃ and the smallest at 16℃ (Fig.1). At the temperature of 24℃ and 28℃, the average shell heights of the OS, SO and SS strains were significantly greater than that of the strain OO ($P<0.05$), while no significant difference was found between the SO and SS strains from day 4 ($P>0.05$). At the temperatures of 16℃, 20℃ and 32℃, the hybrid cross OS showed high growth heterosis (Table 2), and the mean shell height of OS strain was significantly higher than that of the purebred crosses (OO, SS) and the reciprocal cross SO from day 8 ($P<0.05$).

The larval survival rate at temperature 24℃ was higher than those at other temperatures, and the difference was significant ($P<0.05$, Table 3). At the temperatures of 16 and 32℃, the survival rate of purebred strains was lower than reciprocal hybrids. The larvae in OO strain all died on day 12, and in SS strain all died on day 16. Therefore, survival data in these treatments were not included in the statistical analyses. The reciprocal hybrids (OS, SO) at temperature 16 and 32℃ occurred the mass mortality, while both strains survived at all the tested temperatures. Overall, during the whole larval stage, the survival rates of reciprocal crosses (OS, SO) were significantly larger than those of purebred strains (OO, SS) ($P<0.05$). And the survival rate was the highest in OS strain and the lowest in OO strain.

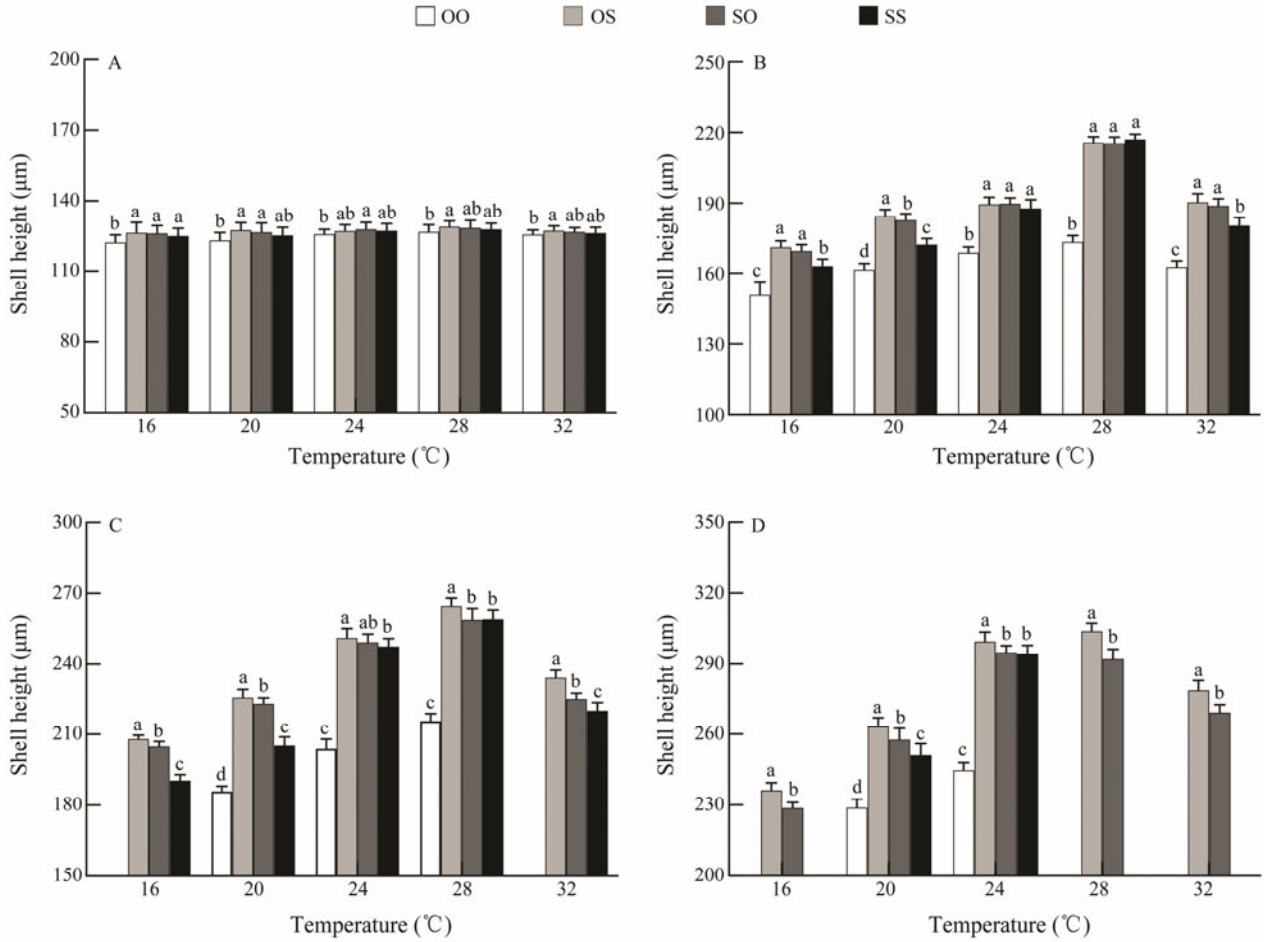

Fig.1 Effects of different temperatures on the larval shell height of the *C. gigas* in different strains on (A) day 4, (B) day 8, (C) day 12 and (D) day 16. Bars denote standard deviation, different letters show significantly different ($P<0.05$).

Table 2 Heterosis for larval shell height (H_{OS} and H_{SO}) in two hybrids crosses of *C. gigas* with different environmental treatments

Environment treatment		H_{OS} (%)				H_{SO} (%)			
		Day 4	Day 8	Day 12	Day 16	Day 4	Day 8	Day 12	Day 16
Temperature (℃)	16	2.18	9.11	–	–	2.02	8.12	–	–
	20	2.65	10.58	15.48	9.68	1.97	9.49	14.13	7.39
	24	0.51	6.31	11.28	11.07	1.10	6.42	10.49	9.43
	28	1.34	10.48	11.45	–	0.95	10.34	9.04	–
	32	1.04	10.95	–	–	0.64	10.11	–	–
Salinity	15	1.82	8.16	15.5	–	1.25	5.37	9.15	–
	20	1.46	7.56	12.10	10.64	0.88	5.33	8.53	8.56
	25	1.25	8.54	8.60	11.94	0.55	3.71	3.89	6.76
	30	1.64	6.89	11.71	11.57	0.88	6.44	10.51	10.09
	35	1.95	16.51	16.38	–	1.57	11.7	10.40	–
Stocking density (larvae mL^{-1})	0.5	2.62	2.14	4.82	5.45	1.88	1.17	3.82	5.07
	1	2.20	1.51	2.03	6.42	2.96	1.42	1.16	5.42
	2	1.46	7.54	11.23	11.04	1.63	6.82	10.52	10.19
	4	5.25	16.69	18.77	–	3.51	14.44	15.88	–
	8	9.43	15.40	–	–	5.03	11.86	–	–

Note: '–' means the value was not available.

Table 3 Effects of different temperatures on the larval survival rate of *C. gigas* in different strains

Temperature (℃)	Strain	Larval survival rate (%)			
		Day 4	Day 8	Day 12	Day 16
16	OO	30.62 ± 1.04^{d}	12.03 ± 1.11^{c}	–	–
	OS	57.21 ± 1.67^{a}	42.47 ± 1.97^{a}	27.55 ± 2.51^{a}	11.64 ± 1.72^{a}
	SO	53.45 ± 2.25^{b}	40.91 ± 2.18^{a}	22.12 ± 2.06^{b}	9.31 ± 1.57^{b}
	SS	44.08 ± 2.20^{c}	28.54 ± 2.24^{b}	11.71 ± 1.65^{c}	–
20	OO	49.47 ± 1.49^{d}	32.25 ± 1.33^{d}	24.42 ± 0.82^{d}	12.00 ± 2.40^{d}
	OS	79.87 ± 3.22^{a}	66.27 ± 1.70^{a}	57.29 ± 2.36^{a}	44.58 ± 2.22^{a}
	SO	70.43 ± 1.87^{b}	62.70 ± 1.65^{b}	50.51 ± 1.95^{b}	39.20 ± 1.77^{b}
	SS	61.27 ± 1.94^{c}	46.93 ± 1.27^{c}	32.13 ± 2.21^{c}	24.32 ± 2.27^{c}
24	OO	71.63 ± 3.03^{d}	43.94 ± 1.25^{d}	31.92 ± 1.98^{d}	23.95 ± 1.94^{d}
	OS	88.43 ± 2.12^{a}	77.76 ± 1.01^{a}	68.61 ± 1.93^{a}	53.57 ± 2.09^{a}
	SO	83.40 ± 2.08^{b}	73.56 ± 1.66^{b}	63.94 ± 2.08^{b}	49.92 ± 2.43^{b}
	SS	75.16 ± 1.85^{c}	60.95 ± 2.01^{c}	47.32 ± 2.44^{c}	29.72 ± 1.90^{c}
28	OO	41.70 ± 3.43^{c}	23.26 ± 1.56^{d}	11.47 ± 1.89^{d}	–
	OS	67.51 ± 2.93^{a}	47.52 ± 1.95^{a}	34.86 ± 1.71^{a}	21.73 ± 2.37^{a}
	SO	62.72 ± 1.45^{b}	41.89 ± 1.89^{b}	28.49 ± 2.80^{b}	11.59 ± 2.29^{b}
	SS	40.56 ± 2.10^{c}	32.19 ± 2.70^{c}	15.15 ± 1.97^{c}	–
32	OO	27.74 ± 1.37^{d}	12.68 ± 1.41^{d}	–	–
	OS	56.89 ± 1.92^{a}	39.09 ± 3.35^{a}	23.41 ± 1.76^{a}	12.99 ± 1.42^{a}
	SO	49.52 ± 1.59^{b}	34.59 ± 2.88^{b}	16.56 ± 2.64^{b}	6.28 ± 1.40^{b}
	SS	38.92 ± 1.79^{c}	25.29 ± 3.39^{c}	5.80 ± 2.05^{c}	–

Notes: Different letters show significant difference ($P<0.05$); '–' means the value was not available.

3.2 Effects of Salinity on Larval Growth and Survival

In the rearing period, the larvae of four strains reared at salinity 25 achieved the highest mean shell height, which was significantly ($P<0.05$) greater than at all the other salinity. There were no significant differences in the mean shell height among the purebred strains and their hybrid crosses on day 4, and the shell height of larvae from the four strains was significantly different from day 8 ($P<0.05$). In all salinity treatments, the shell height of the larvae of OS strain was larger than SO and SS strain, while the OO strain was the smallest (Fig.2).

The larval survival rate of the four strains were significantly affected by salinities ($P<0.05$). The larval survival rate of the four strains were significantly higher at salinity 25 and 30, compared with the other salinity treatments ($P<0.05$, Table 4). In our range of salinity (15–35), all experimental animals in the OS, SO, and SS strains survived, and the survival rate of OS strain significantly outperformed all other three strains, which reveals high heterosis for survival ($P<0.05$, Table 5). Under the optimal salinity 25 in the present experiment, the purebred strain OO showed poor survival performance, which was significantly different from the reciprocal crosses OS and SO ($P<0.05$). At the salinities of 15 and 35, all the larvae from OO strain died on the 16th day.

3.3 Effects of Stocking Density on Larval Growth and Survival

The mean larval shell height of four strains decreased as the stocking density increased. Over the sampling period from day 4 to 16, the larvae reared at the highest density (8 larvae mL^{-1}) had the smallest height, while those at

the lowest density (0.5 larvae mL^{-1}) had the highest height (Fig.3). At low density treatment (0.5 and 1 larvae mL^{-1}), the average shell height of the OS, SO, SS strains was significantly greater than that of the OO strain (P<0.05), while no significant difference was found between the SO and OS strains from day 4 (P>0.05). With high density treatment (4 and 8 larvae mL^{-1}), the average shell height of the OS strain was the highest among four strains, and the significant difference was found between the two reciprocal crosses (OS, SO) (P<0.05).

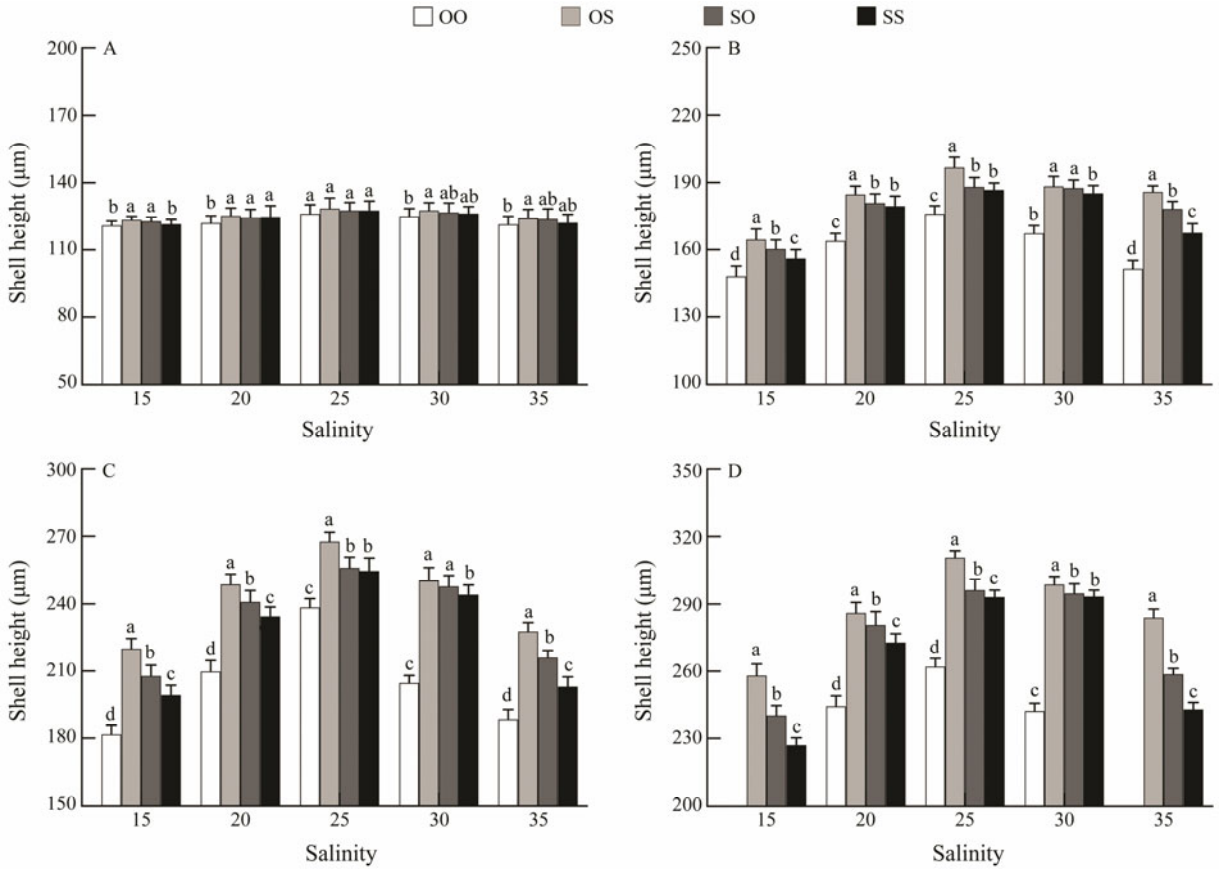

Fig.2 Effects of different salinities on the larval shell height of the *C. gigas* in different strains on (A) day 4, (B) day 8, (C) day 12 and (D) day 16. Bars denote standard deviation, different letters show significantly different (P<0.05).

Table 4 Effects of different salinities on the larval survival rate of the *C. gigas* in different strains

Salinity	Strain	Larval survival rate (%)			
		Day 4	Day 8	Day 12	Day 16
15	OO	32.55±2.70^{c}	13.56±2.65^{d}	5.96±2.27^{d}	–
	OS	55.34±3.18^{a}	45.97±2.59^{a}	32.81±4.08^{a}	22.05±2.73^{a}
	SO	52.81±3.44^{a}	40.12±2.92^{b}	24.63±2.47^{b}	13.30±2.69^{b}
	SS	40.87±3.00^{b}	29.72±2.37^{c}	18.92±2.48^{c}	6.05±1.62^{c}
20	OO	53.05±2.10^{d}	34.18±2.05^{d}	22.49±2.36^{d}	11.46±2.44^{d}
	OS	78.69±2.56^{a}	67.84±2.13^{a}	55.05±2.44^{a}	45.12±3.60^{a}
	SO	73.10±2.32^{b}	58.65±2.41^{b}	44.59±2.10^{b}	33.39±2.58^{b}
	SS	64.15±3.20^{c}	45.03±2.65^{c}	35.10±3.00^{c}	25.07±3.49^{c}
25	OO	63.56±2.48^{c}	49.03±3.06^{c}	36.17±3.38^{d}	30.25±3.12^{d}
	OS	91.73±2.84^{a}	84.13±2.66^{a}	75.48±2.27^{a}	65.14±2.94^{a}
	SO	89.98±3.05^{a}	82.14±2.94^{a}	66.27±3.47^{b}	54.28±3.55^{b}
	SS	84.33±3.10^{b}	63.77±2.97^{b}	53.19±2.50^{c}	40.37±2.43^{c}
30	OO	70.92±3.26^{c}	42.14±3.13^{d}	31.28±2.71^{d}	22.91±3.23^{d}
	OS	87.92±2.49^{a}	74.47±3.51^{a}	71.01±3.27^{a}	54.70±3.54^{a}
	SO	84.52±2.17^{a}	67.17±2.69^{b}	59.41±3.26^{b}	43.23±4.34^{b}
	SS	76.93±2.68^{b}	59.42±3.37^{c}	43.55±3.35^{c}	28.50±3.51^{c}
35	OO	42.62±2.51^{d}	18.73±2.42^{d}	10.33±2.57^{d}	–
	OS	66.70±3.62^{a}	57.70±2.66^{a}	54.84±3.29^{a}	40.55±3.74^{a}
	SO	59.96±2.90^{b}	49.94±3.52^{b}	42.72±2.99^{b}	32.85±4.31^{b}
	SS	47.70±2.89^{c}	33.96±3.33^{c}	20.47±3.48^{c}	11.81±2.64^{c}

Notes: Same as those in Table 3.

Table 5 Heterosis for larval survival rate (H'_{OS} and H'_{SO}) in two hybrids crosses of *C. gigas* with different environmental treatments

Environment treatment		H'_{OS} (%)				H'_{SO} (%)			
		Day 4	Day 8	Day 12	Day 16	Day 4	Day 8	Day 12	Day 16
Temperature (℃)	16	53.17	109.37	370.54	–	43.11	101.68	277.80	–
	20	44.25	67.39	102.62	145.48	27.20	58.37	78.64	115.86
	24	20.49	48.27	73.17	99.63	13.63	40.26	61.38	86.03
	28	64.14	71.40	161.91	–	52.49	51.09	114.05	–
	32	70.69	105.90	707.24	–	48.57	82.20	471.03	–
Salinity	15	50.75	112.43	163.75	628.93	43.86	85.40	97.99	339.67
	20	34.28	71.29	91.18	147.03	24.74	48.09	54.85	82.81
	25	24.05	49.17	68.93	84.48	21.69	45.64	48.32	53.72
	30	18.93	46.65	89.79	112.80	14.33	32.28	58.79	68.18
	35	47.70	119.02	256.10	586.71	32.77	89.56	177.40	456.31
Stocking density (larvae mL^{-1})	0.5	4.08	4.69	9.88	6.56	2.67	3.12	5.53	1.70
	1	5.28	10.47	13.74	6.00	2.68	5.80	5.49	3.29
	2	22.64	49.17	83.62	100.40	11.59	37.00	59.82	72.27
	4	45.00	59.85	118.81	365.69	30.04	41.82	75.29	210.46
	8	67.01	173.72	–	–	44.60	109.54	–	–

Note: '–' means data are not available.

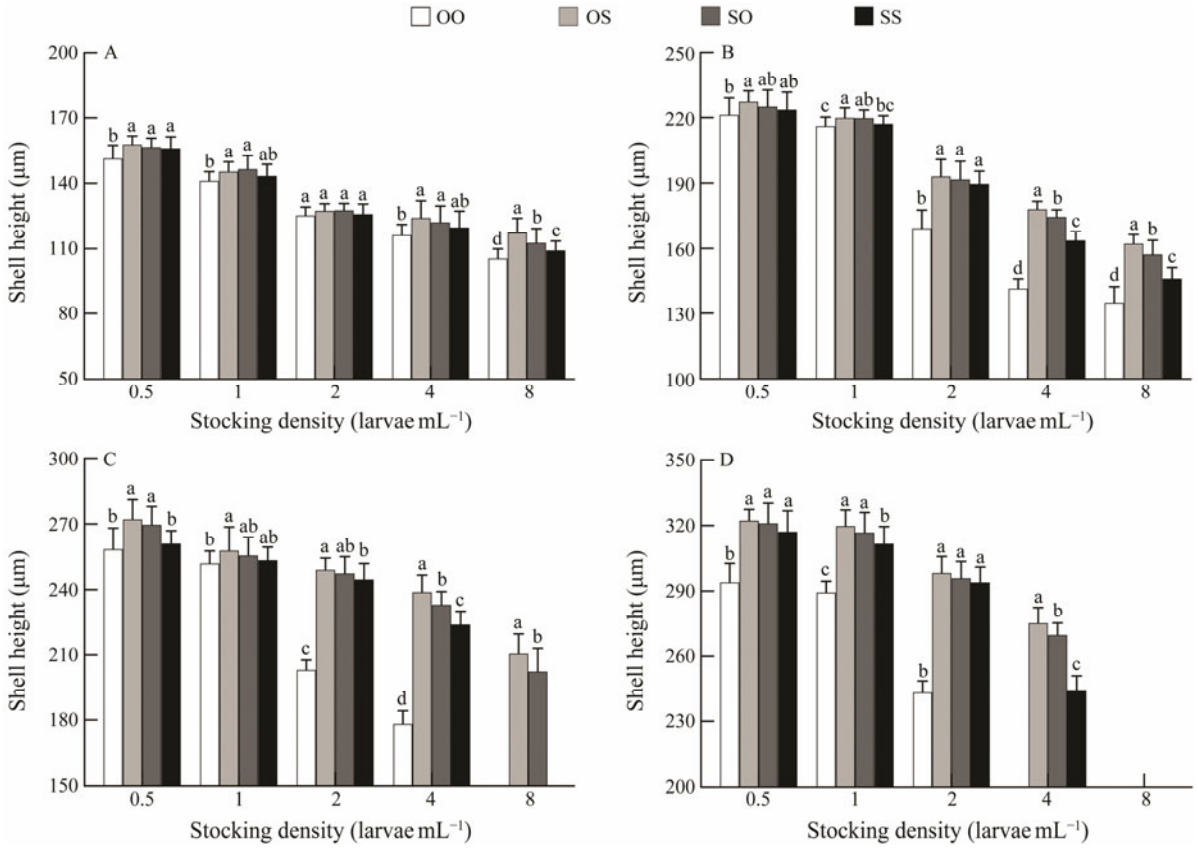

Fig.3 Effects of different stocking density on the larval shell height of the *C. gigas* in different strains on (A) day 4, (B) day 8, (C) day 12 and (D) day 16. Bars denote standard deviation, different letters show significantly different ($P<0.05$).

From day 4, the larval survival rates of four strains with low density treatments (0.5, 1 and 2 larvae mL^{-1}) were significantly ($P<0.05$) higher than those with high density treatments (4 and 8 larvae mL^{-1}) (Table 6). On day 4, survival rate of larvae from four strains remained above 65% with an increase in stocking density from 0.5 to 2 larvae mL^{-1}; however, a further increase in density of 4 and 8 larvae mL^{-1} caused a reduction in survival. The larvae in purebred strains OO and SS reared at 8 larvae mL^{-1} died at day 12 of the experiment, and the survival rate of OS strain was higher than that of SO strain. On the 16th day of the experiment, all the reciprocal crosses (OS, SO) reared at 8 larvae mL^{-1} had died. Therefore, survival data from these treatments were not included in the statistical analysis. In the rearing period, the OS strain showed obvious survival heterosis, and on the 16th day of feeding, the survival rate at 2 larvae mL^{-1} was higher than 50% (as shown in Table 6).

Table 6 Effects of different stocking densities on the larval survival rate of the *C. gigas* in different strains

Stocking density (larvae mL^{-1})	Strain	Larval survival rate (%)			
		Day 4	Day 8	Day 12	Day 16
0.5	OO	87.63±3.05^{b}	84.95±2.74^{b}	72.41±2.22^{c}	73.04±3.34^{b}
	OS	91.73±2.61^{a}	90.03±1.73^{a}	83.41±3.24^{a}	78.77±2.84^{a}
	SO	90.49±1.39^{ab}	88.68±1.96^{a}	80.11±1.47^{ab}	75.18±2.42^{ab}
	SS	88.64±3.30^{ab}	87.05±2.42^{ab}	79.41±3.01^{b}	74.80±2.91^{ab}
1	OO	86.11±2.45^{b}	80.23±3.03^{b}	70.18±2.78^{c}	70.88±1.80^{b}
	OS	90.66±3.03^{a}	88.63±1.43^{a}	79.82±1.51^{a}	75.13±2.55^{a}
	SO	90.05±3.30^{a}	86.49±2.46^{a}	76.21±3.42^{ab}	73.45±2.18^{ab}
	SS	89.29±1.68^{ab}	83.26±1.89^{b}	74.31±3.25^{b}	71.34±2.77^{b}
2	OO	67.24±3.35^{c}	42.25±3.03^{d}	32.62±3.33^{d}	22.87±2.43^{d}
	OS	87.13±3.01^{a}	76.60±3.41^{a}	70.61±3.39^{a}	55.36±2.81^{a}
	SO	79.28±3.25^{b}	70.35±4.08^{b}	61.46±2.38^{b}	47.59±3.90^{b}
	SS	74.85±3.84^{b}	60.45±2.88^{c}	44.29±3.37^{c}	32.38±1.88^{c}
4	OO	39.04±3.13^{d}	24.52±3.77^{d}	8.88±2.16^{d}	–
	OS	61.14±2.16^{a}	44.43±3.57^{a}	27.80±1.38^{a}	12.69±1.71^{a}
	SO	54.83±3.93^{b}	39.42±2.59^{b}	22.27±2.35^{b}	8.46±1.61^{b}
	SS	45.29±3.40^{c}	31.07±2.64^{c}	16.53±1.37^{c}	5.45±1.55^{c}
8	OO	23.67±2.87^{d}	8.18±2.25^{d}	–	–
	OS	44.95±3.05^{a}	28.70±3.16^{a}	11.21±1.38^{a}	–
	SO	38.92±3.01^{b}	21.97±2.54^{b}	5.54±1.10^{b}	–
	SS	30.16±3.07^{c}	12.79±3.38^{c}	–	–

Notes: Same as those in Table 3.

4 Discussion

4.1 Temperature

The growth performance of oysters was affected by temperature, especially at the upper (32℃) and lower (16℃) levels where significant differences in the shell height and survival rate were detected ($P<0.05$). In our study, increasing temperature accelerated the larval development. The rapid growth of *C. gigas* larvae in the higher temperature range coincided with the results obtained from other bivalve species such as *Mytilus galloprovincialis* (Lazo and Pita, 2011), *Saccostrea echinate* (Nowland *et al.*, 2019) and *Macoma balthica* (Drent, 2002). As a general rule, an increase in water temperature augments the activity and accelerates the metabolic processes to promote the growth of larvae. On the other hand, a higher mortality rate was caused by the increasing temperature. Due to the fast reproduction of bacteria and other microorganisms in the higher temperature ranges, more oxygen is consumed by these microorganisms and less oxygen can be used by the larvae for their growth, which causes mass mortality in bivalve hatcheries (Gruffydd and Beaumont, 1972). The mortality of larvae at temperature 32℃ is in agreement with the findings of most bivalve larval studies. For example, Nair and Appukuttan (2003) found that there was complete mortality after 24 h of the larvae reared at 33℃ and 35℃. Moreover, the survival rate at high temperature (32℃) were much lower than the results of the previous studies about *C. gigas*. Kheder *et al*. (2010) found the survival rate at the end of larval development was always above 90% at all trial temperatures (17–32℃). The results may be explained by geographical factors, as the *C. gigas* in subtropical and tropical areas can have different adaptabilities to temperature change (Eirman and Hare, 2013). The lowest temperature applied in the present study (16℃) caused a significant reduction of larval growth, and the mortalities were ≥40% after 4 days of rearing. The inability of larvae to grow at low temperature could be due to their inability to digest ingested microalgae (Nair and Appukuttan, 2003). The higher values for shell height and survival rate were found in *C. gigas* that were held at 24℃, indicating 24℃ is the optimum growth and survival temperature for larval *C. gigas*.

4.2 Salinity

It is well known that salinity is a major environmental parameter and the fluctuation in salinity is the major factor to change the distribution of bivalves. This study demonstrated that these four strains of larval *C. gigas* could grow within a salinity range of 15–35. The highest shell height and survival rate were found in *C. gigas* that was held at salinity 25, indicating this as the optimum salinity for larval *C. gigas*. Moreover, larval growth and survival were positively correlated with increasing salinity up to 25, from which further salinity increases resulted in declining larval shell height and survival rate. The results of the present study have shown that lowering salinity during larval culture had significantly positive effects on larval development. Other oyster larvae that display a preference for low salinity include *Crassostrea nippona*, while the larvae achieved the highest mean shell and maximum survival at salinity 26 (Wang *et al*., 2018). Another example is *Crassostrea hongkongensis*, as the hatchery and nursery culturing of this species at lower salinities was recommended to produce a higher yield (Huo *et al.*, 2014). At the lowest salinity 15 and the highest salinity 35, the minimum shell height and the lowest survival rate were observed during the rearing period. Previous studies showed that, increased excretion of ammonia and decreased free amino acid con-

centration at low salinity led to lower energy for growth (Pourmozaffar *et al*., 2019). Additionally, food assimilation and feeding efficiency reduced when salinity is beyond the toleration range (Wang *et al*., 2011).

4.3 Stocking Density

In the present study, the shell height and survival rate remained consistent across stocking density at 0.5 and 1 larvae mL^{-1}, decreasing significantly at the other tested densities of 2, 4, 8 larvae mL^{-1}. The result indicated that poor growth performance and higher mortality were observed at densities greater than 4 larvae mL^{-1}. High stocking density has been reported to negatively affect larval growth and survival in many bivalves (Daume *et al.*, 2004; Li and Li, 2010; Ren *et al*., 2017). Firstly, the limited growth of shell height at high densities is partly due to the intraspecific competition for food and space. The per capita food supply for high-density individuals is lower than that for low-density individuals (Hadley and Manzi, 1984). In our research, food should not be a limiting factor because algal concentrations were adjusted according to larval densities to ensure that larvae in each treatment received an equal diet. At high densities, physical contact between individuals can inhibit the food intake, while shells may suffer damage and more energy is allocated to repair this damage (Holliday *et al.*, 1991). Secondly, the high mortality of larvae rearing at high densities can also be caused by poor water quality. With increasing stocking densities, more metabolic wastes will be accumulated in the water, resulting in low oxygen level and high ammonia content (Deng *et al*., 2013). Although daily water changes with filtered, UV-sterilized seawater were made to minimize bacterial colonization, it is still inevitable at high density. In addition, when *C. gigas* larvae were reared at 0.5 and 1 larvae mL^{-1}, there was no statistically significant difference in survival rate. Results demonstrated that survival rate was independent of density when larvae were reared at optimal densities. Similar results also occurred in other studies. For example, Liu *et al.* (2006) reported that no correlation has been found between larval survival rate (74.8%−79.1%) and density (5, 10, 20, 40, 60 ind mL^{-1}) in the treatments. As mentioned previously, high stocking density can cause higher larval mortality while lower stocking density can achieve rapider growth, a moderate density ranging from 1 to 2 larvae mL^{-1} is recommended for *C. gigas* larval hatchery production to achieve rapid growth, high survival rate and maximum economic benefit.

4.4 Heterosis

The objective of selective breeding is to achieve a stable state of excellent production characteristics in different environments, that is, a variety of offspring should have the properties that can regulate its performance, adapt it to different environments, and maintain its inherent balance of physiological and reproductive traits. In the present study, the selected strain had a greater growth performance and survival ability compared to the orange shell variant. It has documented that the selected strain has an average increase in growth of 10% per generation than general oysters (Li *et al*., 2011). Furthermore, the orange shell variant showed similar performance at optimal conditions and potential disadvantages in suboptimal conditions compared to the general oysters (Han and Li, 2018). Heterosis for growth and survival has been demonstrated in crossbreeding of inbred *C. gigas*, and the yield increased with general diallel crosses among inbred parent lines (Hedgecock *et al*., 1995; Hedgecock and Davis, 2007). According to our data, the hybridization can promote the growth and survival of oysters, in which the shell height and survival rate of the two reciprocal crosses (OS, SO) were significantly greater than those of the two purebred strains (SS, OO) in all treatments ($P<0.05$). In particular, OS strain showed greater heterosis compared to the purebred strains and SO progeny as well. In contrast, other similar studies reported that the hybrid larvae showed intermediate growth between the parental species, which was reported for *C. hongkongensis*×*C. ariakensis* (Huo *et al.*, 2013), *C. gigas*×*C. rivularis* (Allen Jr. and Gaffney, 1993) and *Argopecten irradians concentricus* Say×*A. Irradians irradians* Lamarck (Zhang *et al.*, 2007). In addition, in many interspecific hybrids, the progeny exhibits slower growth than the parent species (Beaumont *et al*., 2004; Zhang *et al.*, 2012). This study showed undoubtedly the high tolerance of the reciprocal cross OS larvae under extreme environments. If bivalves can tolerate a wide range of environmental fluctuation, it is possible that they become dominant species under challenging environmental conditions.

5 Conclusions

This study firstly examined larval growth and survival of reciprocal crosses between two strains of *C. gigas* at different environmental conditions. Results indicated that the larval productive traits of the selected strain 'Haida No. 1' could be improved by crossing with the orange shell line. Under different environments, the hybridization between the 'Haida No. 1' and the orange shell variant showed the heterosis of growth and survival. The reciprocal cross OS reveals higher heterosis for growth and survival compared to the reciprocal cross SO. The environmental parameters, including the temperature of 24℃, the salinity of 25, the stocking density of 2 larvae mL^{-1} in present study, provide optimal culture conditions for *C. gigas* in the hatchery. These findings can guide future hatchery breeding of new variety with high survival rate.

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